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TITLE: Does the Androgen Receptor (AR)-Regulated Map Kinase Phosphatase 1 (MKP-1) Enhance Castration-Resistant Prostate Cancer Survival under Therapeutic Stress?

PRINCIPAL INVESTIGATOR: Russell Szmulewitz

CONTRACTING ORGANIZATION: University of Chicago
Chicago, IL 60637

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Russell Szmulewitz

E-Mail: rszmulew@uchicago.edu

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University of Chicago
5841 S. Maryland Avenue,
MC 2115
Chicago, IL 60637

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Metastatic castration resistant prostate cancer (mCRPC) is clinically treated with both taxane chemotherapy and androgen pathway modulators. Identification of a mediator of resistance across therapy classes is a critically unmet need and would be a significant innovation in the field. Map Kinase Phophatase 1 (MKP-1, DUSP1) is a known regulator of the stress activated protein kinase cascade that can inhibit the activity of pro-apoptotic Map Kinases JNK and p38. It has an established anti-apoptotic, pro survival role, and has been implicated in chemotherapy resistance in breast cancer models, and is inversely associated with apoptosis in preclinical prostate cancer models. Androgen and glucocorticoid signaling can induce MKP-1 expression; as mCRPC remains driven by androgen receptor signaling, and as mCRPC is often treated adjunctively with corticosteroids, MKP-1 may be a down stream effector of prostate cancer cell survival that facilitates therapy resistance. The work proposed sought to test the hypothesis that MKP-1 plays a role in the development of therapy resistance, independent of therapeutic class, and thus, if inhibited, would potentiate the effects of both hormonal and chemotherapies.

To date, significant progress has been made including optimization of MKP-1 protein detection methodologies and the generation of highly bone metastatic CRPC cell lines that can be traced over time with bioluminescence imaging. Ongoing experiments are being undertaken with MKP-1 over expression and knock-down within these cell lines to test for *in vivo* therapy resistance.

15. SUBJECT TERMS

Metastatic castration resistant prostate cancer (mCRPC), Prostate Cancer (PC), Enzalutamide (Enza), Map Kinase Phophatase 1 (MKP-1)

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Table of Contents

1 INTRODUCTION:

Metastatic castration resistant prostate cancer (mCRPC) is clinically treated with both taxane chemotherapy and androgen pathway modulators. Identification of a mediator of resistance across therapy classes is a critically unmet need and would be a significant innovation in the field. Map Kinase Phophatase 1 (MKP-1, DUSP1) is a known regulator of the stress activated protein kinase cascade that can inhibit the activity of pro-apoptotic Map Kinases JNK and p38. It has an established anti-apoptotic, pro survival role, and has been implicated in chemotherapy resistance in breast cancer models, and is inversely associated with apoptosis in preclinical prostate cancer models. Androgen and glucocorticoid signaling can induce MKP-1 expression; as mCRPC remains driven by androgen receptor signaling, and as mCRPC is often treated adjunctively with corticosteroids, MKP-1 may be a down stream effector of prostate cancer cell survival that facilitates therapy resistance. The work proposed sought to test the hypothesis that MKP-1 plays a role in the development of therapy resistance, independent of therapeutic class, and thus, if inhibited, would potentiate the effects of both hormonal and chemotherapies.

2 KEYWORDS

The following are key words that will be used in this report:
Metastatic castration resistant prostate cancer (mCRPC)
Prostate Cancer (PC)
Enzalutamide (Enza)
Map Kinase Phophatase 1 (MKP-1)

3 ACCOMPLISHMENTS:

A. What were the major goals of the project?

Objectives: To demonstrate that decreased MKP-1 expression enhances response to androgen targeted therapy (Aim #1) and docetaxel chemotherapy (Aim #2) and delays therapy resistant progression in a preclinical model of metastatic CRPC.

As described in the statement of work, these were the outlined goals of the project

Task 1. **Generation and optimization of cell lines**

a. Develop prostate cancer cell lines lines stably expressing luciferase construct for use in bioluminescence
b. Develop cell lines that express doxycycline inducible MKP-1 knockdown
c. Confirm that cell lines, when exposed to doxycycline *in vitro* display knockdown of MKP-1

Task 2: Establish progressive mCRPC *in Vivo*

a. Perform intracardiac injections to disseminate prostate cancer cells systemically
b. Follow animals for development of metastatic disease using live animal bioluminescence imaging
c. Castrate animals upon development of metastatic disease
d. Follow for the development of castration resistance by bioluminescence imaging

Task 3: Treatment with MDV3100, docetaxel chemotherapy with/without MKP-1 knockdown

Upon development of castration resistance, separate mice into two cohorts-one for MDV3100 hormonal treatment and one for docetaxel treatment

a. Treat animals with docetaxel or MDV3100 for 4 weeks. Half the mice will also receive diet containing doxycycline to induce MKP1 knockdown
b. Monitor metastatic disease progression with bioluminescence while on treatment until disease endpoint

Task 4: Pathologic evaluation of metastatic tissues:

a. Harvest tissue from euthanized mice
b. Send to tissue resource center for fixation/processing/staining
c. Evaluate the tumor tissue for histologic characterization, staining for proliferation and apoptosis.

B. What was accomplished under these goals?

Although not all of the tasks were completed (see Section 5), significant progress was made on many of the tasks.

Task 1 focused on the development of cell lines for use in the experiments. Cell lines expressing luciferase have been made (see Figure 2). The baseline MKP-1 RNA/protein expression for our cell lines of interest has been assessed (Figure 1). Cell lines that over-express MKP-1 conditionally have been made and are in the process of validation (Figure 1). Task 2 is complete. The cell lines initially proposed (C4-2B and CWR 22Rv11) were utilized for our metastatic model initially. However, the C4-2B cell line generated no distant metastases subsequent to intracardiac cell inoculation. The 22Rv1 cell line did establish metastases, however the metastatic yield was low with this line. We therefore optimized new cell lines for our experimental metastasis assay. Two cell lines were generated to be resistant to the androgen receptor antagonist enzalutamide (Enza-R). Enza-R LNCaP cells and CWR-R1 cell lines demonstrate significant castration resistant metastatic progression (Figure 2). Of note, the C4-2 line is a derivative of the LNCaP line, and the CWR-22Rv1 and CWR-R1 lines are similarly genetically related. Thus although the cell lines we are utilizing are different from those proposed, they are genetically and phenotypically very similar.

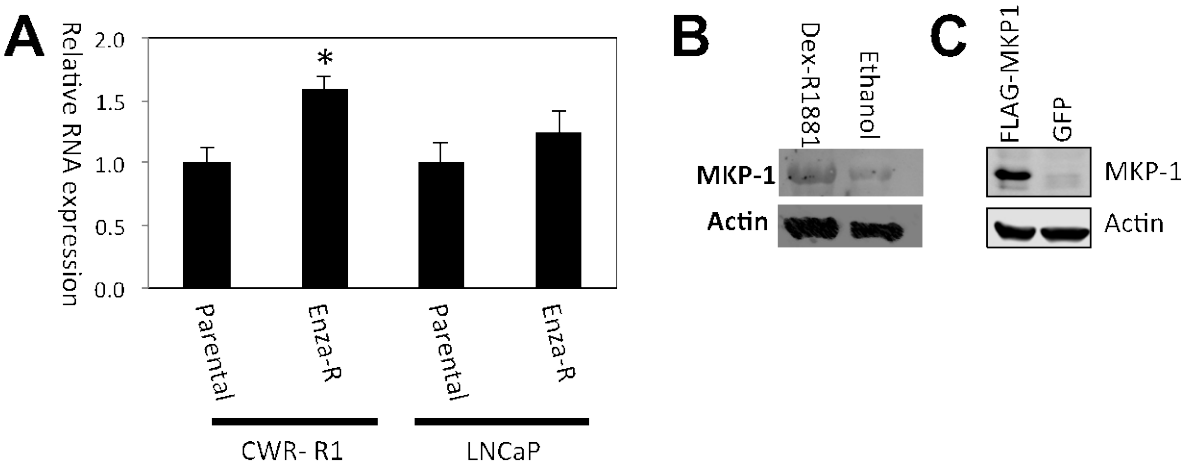
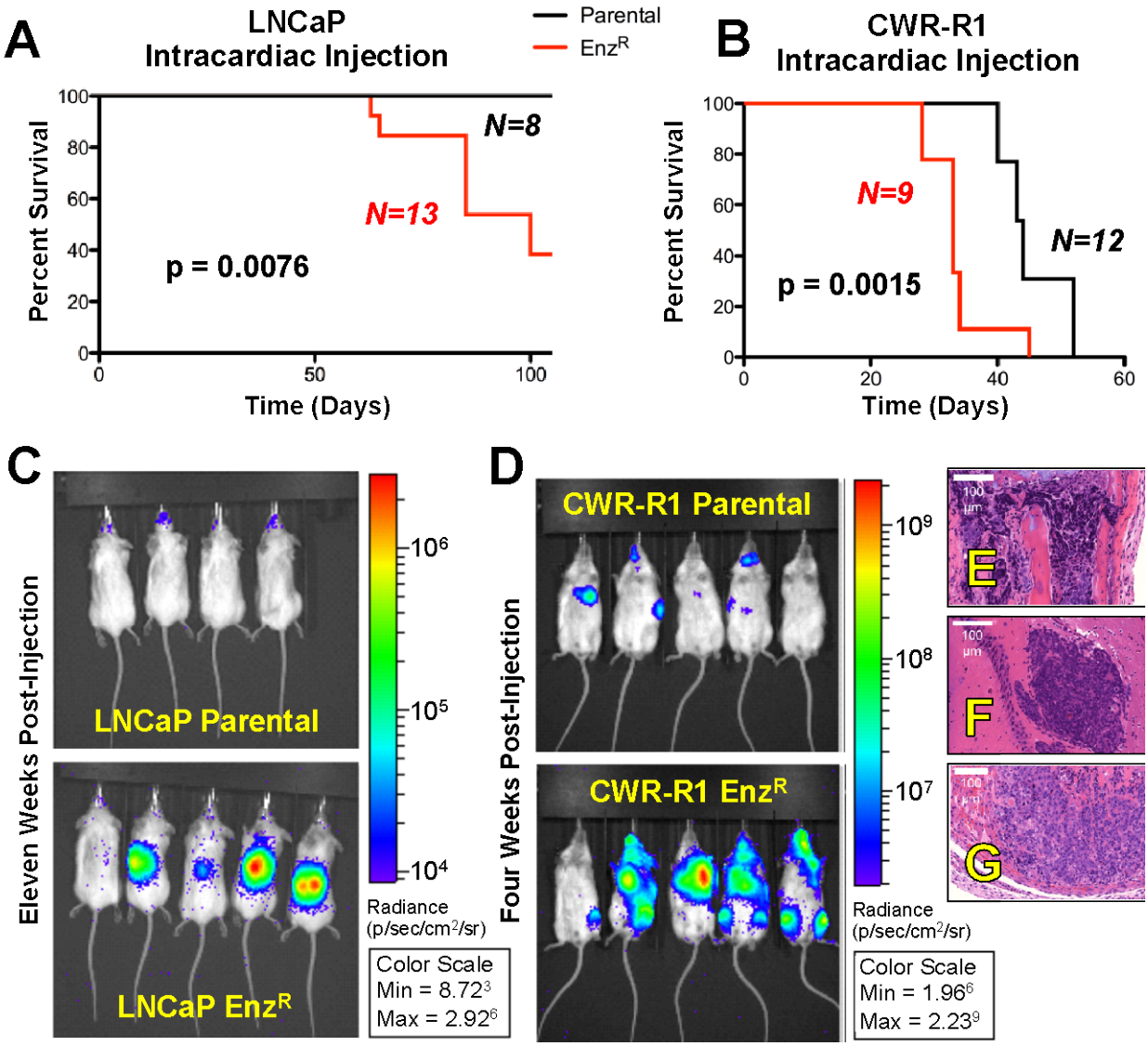


Figure 1. MKP-1 expression in PC lines. A. Endogenous *MKP-1* RNA expression by qRT-PCR showing increased expression in Enza-R CWR-R1's (* P<0.05) and stable levels in LNCaP despite enzalutamide treatment. B. MKP-1 protein in LNCaP-Enza-R induced with dexamethasone (Dex) and androgen (R1881). C. Successful FLAG-MKP1 transfection in lentiviral generating 293T cells.

Figure 2. Metastatic model optimization. Enzalutamide-resistant (Enza-R) cell lines were derived through prolonged culture in enzalutamide-containing media. 5 x 10^5 (LNCaP, A) or 2.5 x 10^5 (CWR-R1, B) parental or Enza-R luciferase expressing cells were inoculated via intracardiac injection. Animal survival was significantly shorter with Enza-R compared to parental cells for both lines. Metastatic disease was more frequent and with higher volume as denoted by bioluminescence (C, D). Representative histology of bone (E), brain (F) and adrenal gland (G) CWR-R1 Enza-R metastases.



Task 3 has not yet been completed (see section 5 for reasons for delay).

For Task 4, progress has been made, however completion of the task is dependent on Task 3. As prostate cancer is often bone metastatic, task 2 was focused on optimizing a metastatic model that spread within the bones. As noted this has been accomplished. As a necessary byproduct of this model, we have needed to optimize models for identifying, obtaining and decalcifying bone metastases for histopathologic examination within this task. This has been accomplished (Figure 2 for example).

C. What opportunities for training and professional development has the project provided?

Not applicable

D. How were the results disseminated to communities of interest?

No final results have reported during this reporting period. A preliminary report of the derivation of the Enza-R metastatic cell lines was reported at national meetings (AACR Prostate Cancer meeting and ASCO genitourinary symposium) in 2014.

E. What do you plan to do during the next reporting period to accomplish the goals?

As the metastatic models have now been optimized (section 3B) and cell lines generated, we will in the next reporting period conduct the seminal experiments outlined above. Specifically, the following tasks will be completed:

Task 1: Although MKP-1 over-expressing cells have been generated, conditional knockdown cell generation is underway, now that a reproducible antibody for protein detection has been optimized.

Task 2: Completed

Task 3: The entirety of this task will be completed during the subsequent reporting period. Specifically, the over-expressing MKP-1 cells (and controls) will be inoculated into castrated mice and distant metastases formed. Upon development of metastases, animals will be randomized to either enzalutamide or docetaxel treatment. Differences in resistance to therapy will be monitored with bioluminescence imaging. In addition, cell lines with the conditional knock-down of MKP-1 will be derived now that MKP-1 protein assessment is feasible and the cell line models have been validated. Similar in vivo experiments with these cell lines will be completed.

Task 4: This task is dependent on Task 3. However, we have optimized methods within our lab for tissue processing from the bone (see figure 2) that allow histopathologic assessment of tissues that will be gathered upon completion of Task 3.

4. IMPACT:

A. What was the impact on the development of the principal discipline(s) of the project?

As the project is not yet complete, the full impact is not yet known. However, the generations of Enza-R cell lines that are highly metastatic, especially to the bone, is a novel and highly impactful contribution to the prostate cancer field. We are preparing the manuscript detailing these lines for publication in the near term. We will also publish our bone histology techniques, which will have an impact on the prostate cancer field.

B. What was the impact on other disciplines?

Surprisingly, endogenous MKP-1 protein analysis by Western blot has not been routinely reported in the literature, likely due to difficulties with commercially available antibodies. We have optimized techniques using one of these antibodies, which significantly increases specificity and sensitivity of endogenous MKP-1 protein detection. This will have an impact beyond the prostate cancer field for others studying MKP-1.

C. What was the impact on technology transfer?

None

D. What was the impact on society beyond science and technology?

None at this point. Depending on the results, the work may lead to new therapy targets in prostate cancer.

5. CHANGES/PROBLEMS:

A. Changes in approach and reasons for change

As noted in Section 4B, we changed the prostate cancer cell lines we will use (have used) in this work. The reasons for this change are noted above, but restated, the initially selected cell lines did not reproducibly produce a metastatic phenotype, despite their report in the literature. The cell lines we are using now reproducibly metastasize. Another change involves the use of MKP-1 over-expressing cell lines. We initially proposed conditional MKP-1 knockdown, however, as endogenous MKP-1 protein expression has been difficult to detect, we engineered cell lines to over-express flag-MKP-1. The central hypothesis remains intact and we will begin testing these cell lines for enhanced therapy resistance. As we now can detect endogenous MKP-1 (required antibody optimization), MKP-1 knock-down cell lines are being generated for use as initially outlined as well. This in total, will allow more complete assessment of MKP-1's role in mCRPC therapy resistance.

B. Actual or anticipated problems or delays and actions or plans to resolve them

There were several delays/problems in the culmination of the work over the past year.

1. The Postdoctoral Fellow who was to lead the day to day effort on the work, with PI supervision, left the institution 5 months into this award period. This caused a significant delay in the progress of the project. A new postdoctoral fellow has been hired and tasked with completion of the work. This is the major reason for delay in completion of the project and we have been granted an extension of the award period to finish the tasks proposed.
2. The metastatic capacity of the cell lines proposed was less then anticipated/reported in the literature (see sections 3B and 5A for details). Two alternative prostate cancer cell lines were developed with greatly enhanced metastatic capacity that will be used for the entirety of the work.
3. The antibodies reported to detect MKP1 protein were not satisfactorily consistent in their specificity or sensitivity. Multiple antibodies were screened and we now have methods to optimize one specific antibody for consistent MKP1 protein level analysis (see section 5B).

C. Changes that had a significant impact on expenditures

None

D. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: None

E. Significant changes in use or care of human subjects: None

F. Significant changes in use or care of vertebrate animals: None

G. **Significant changes in use of biohazards and/or select agents:** None

6. PRODUCTS:

A. Publications, conference papers, and presentations:
The metastatic model (Task 2) was presented at GU ASCO 2014 and AACR Prostate symposium 2014.

B. Website(s) or other Internet site(s): NA

C. Technologies or techniques: Technique for optimization of MKP-1 protein detection was developed and will be reported in next manuscript. A new metastatic model was developed (see above).

D. Inventions, patent applications, and/or licenses: NA

E. Other Products: NA

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

A. What individuals have worked on the project?

Other then the principal investigator, the postdoctoral fellow (first Erin Howe and now most recently Jacob Kach) have worked on the project. In addition, a graduate student, Steve Kregel, has been peripherally involved in the project, assisting with live animal imaging and in vivo studies. The animal experiments were performed in collaboration with Dr. Donald Vander Griend.

B. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? None

C. What other organizations were involved as partners? None

8. SPECIAL REPORTING REQUIREMENTS

None

9. APPENDICES

None